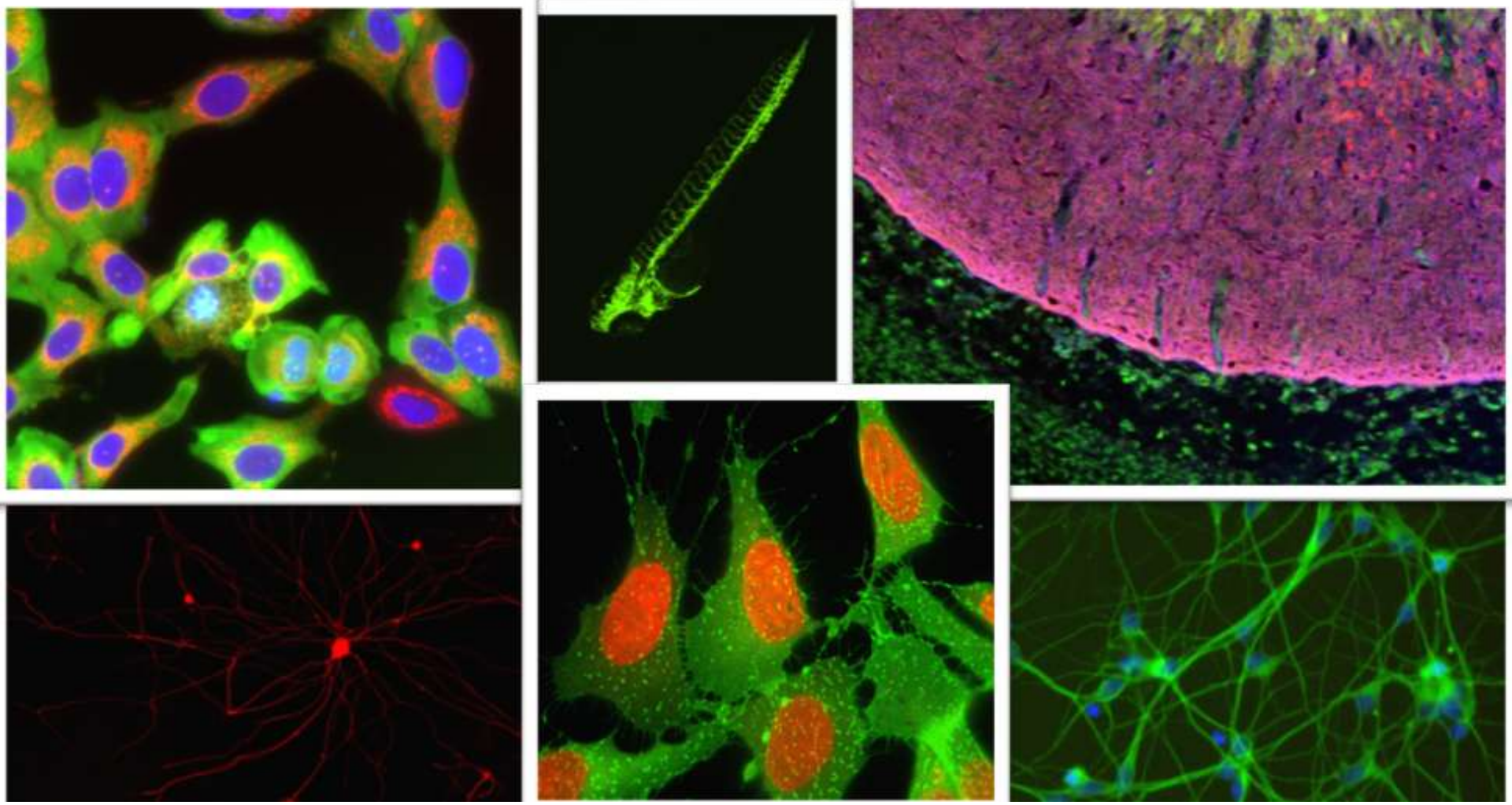


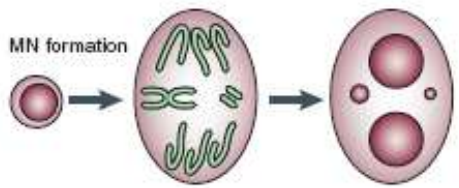
Molecular Devices

Together through life sciences.

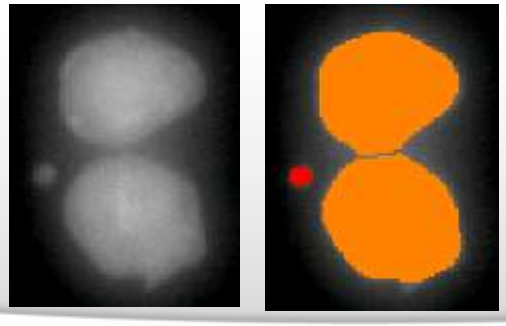
MetaXpress® Software:
Micronuclei Module

Micronuclei Biology

- Micronuclei are small nuclei produced during cell division by a lagging chromosome fragment or entire chromosome



- Micronuclei induction is a highly quantitative measurement of chromosomal damage.
- Enables screening for indicators of genetic toxicity early in the development of therapeutic candidate



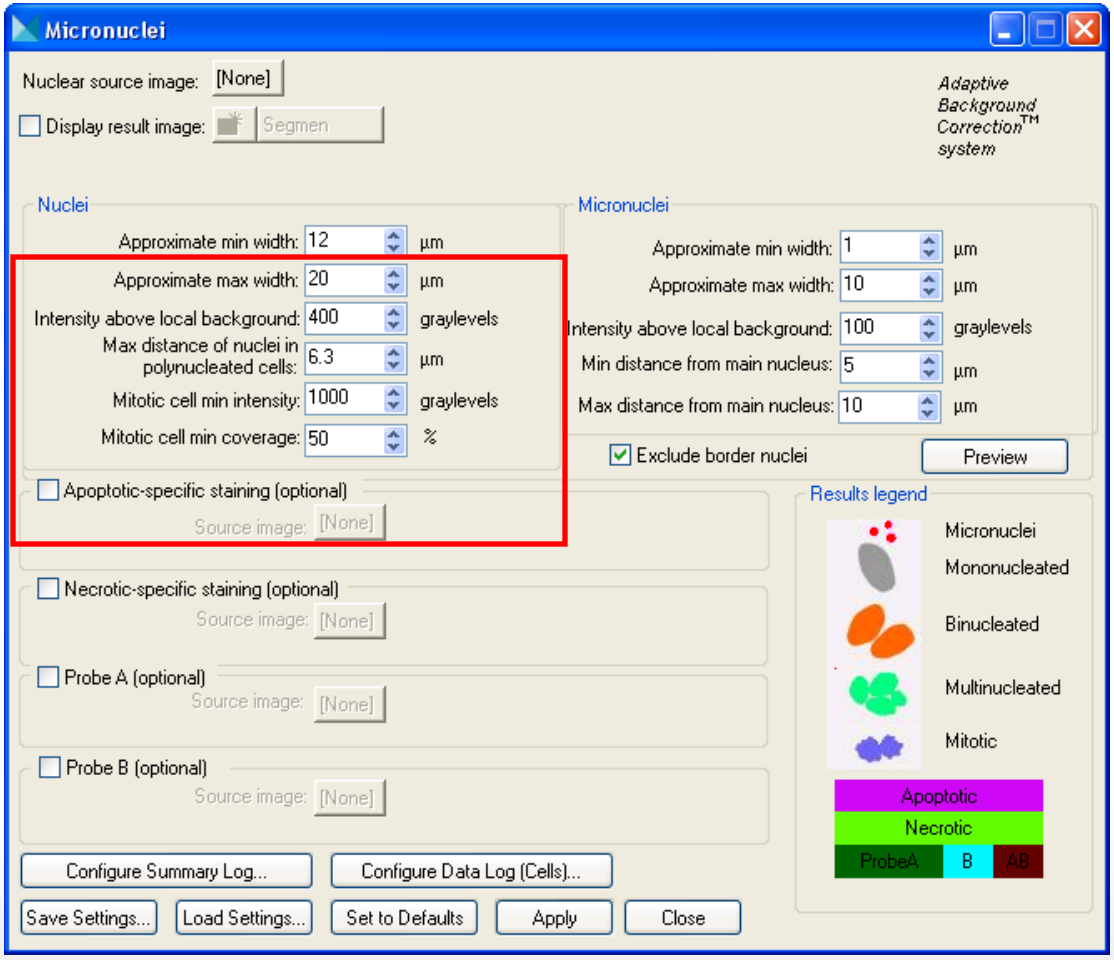
MetaXpress® Software Micronuclei Application Module

- Only requires one wavelength (nuclear stain)
  - Reduces sample preparation, image acquisition and analysis time
  - 4 additional probes (Apoptosis, Necrosis + 2 custom)
- Classification of multi-nucleated cells is highly accurate
  - Proprietary algorithm
  - Discriminate phenotypes based on Cell Morphology, number of nuclei, Distance of Micronuclei from Nucleus, Micronuclei vs. “Blebs” or “buds”
- Multiple measurement outputs
  - 68 parameters per image
  - 30 parameters per cell

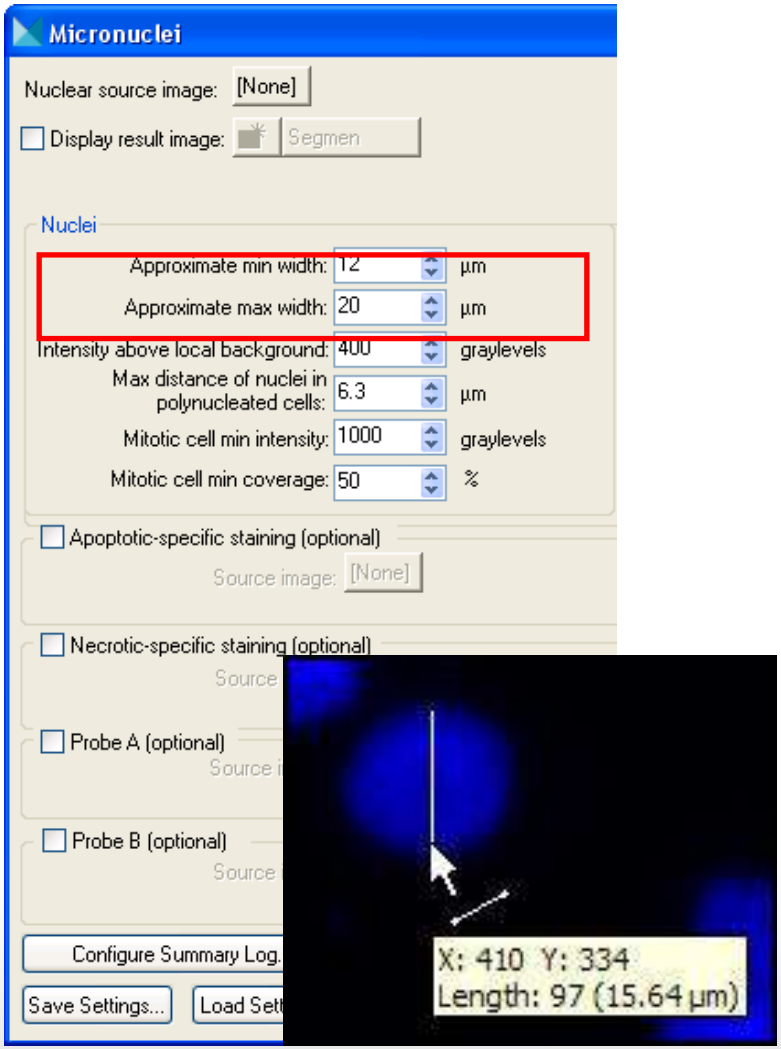
Scoring of Micronuclei

- Micronuclei are morphologically identical to but smaller than nuclei: between 1/16th and 1/3rd of the mean diameter of the main nuclei
- Micronuclei are not linked or connected to the main nuclei; they may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary.
  - Application module allows setting up a min and max distance from main nucleus
- Micronuclei usually have the same staining intensity as the main nuclei but occasionally staining may be more intense.
  - Application module allows to set-up an intensity threshold for Micronuclei

1. Detect Mono- and Poly-nucleated + Mitotic Cells



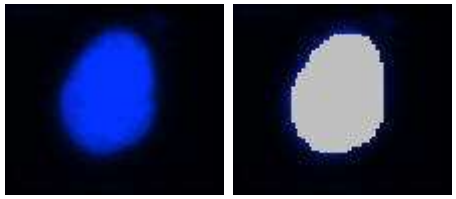
Module Settings



- **Nuclear stain**
- **Set the Approximate min width** and **Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in um)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

Module Settings

Effects of varying width settings



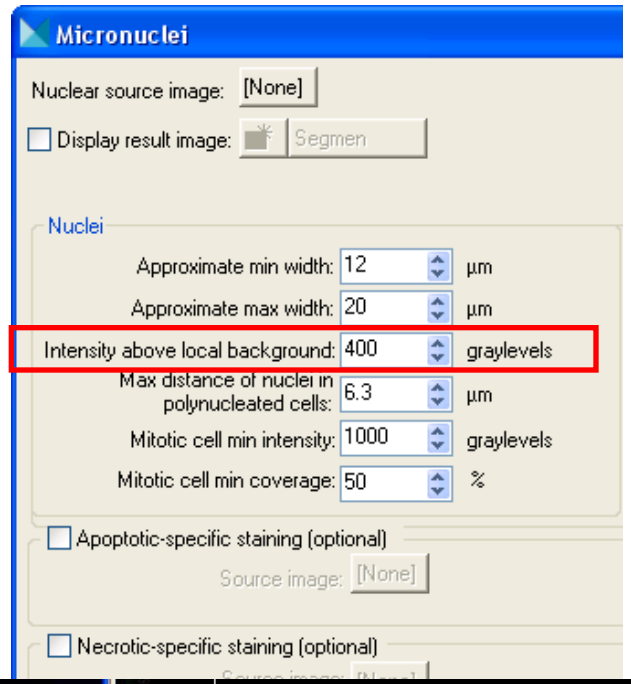
Min width too small: splits nuclei

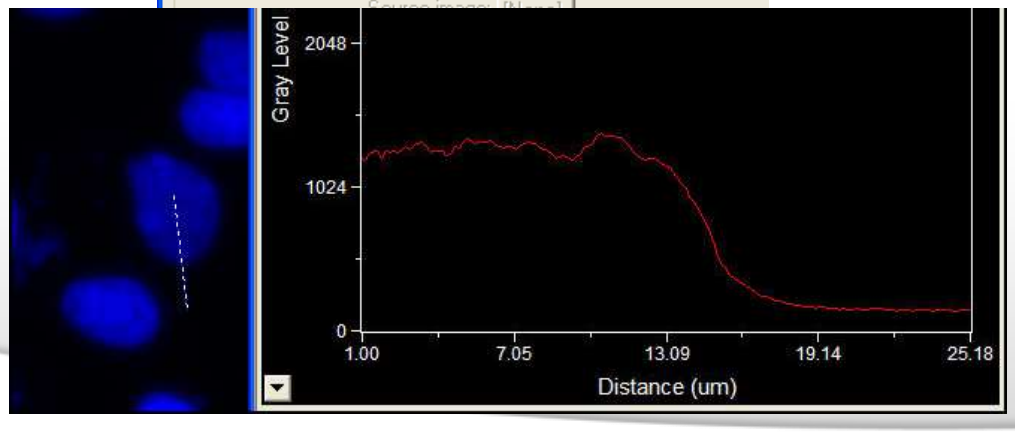
Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries

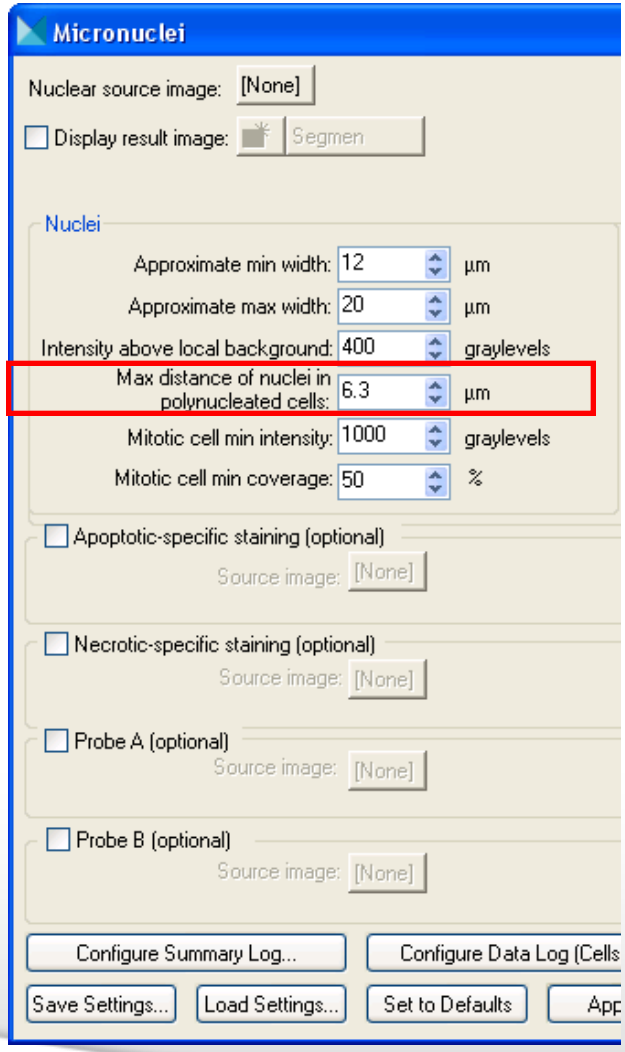
Module Settings





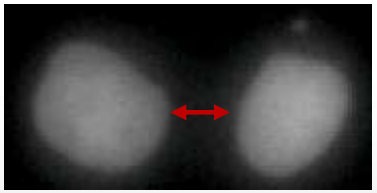
- **Nuclear stain**
- The **intensity above local background** is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background
- Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the cell and the background and view the intensity values

Module Settings

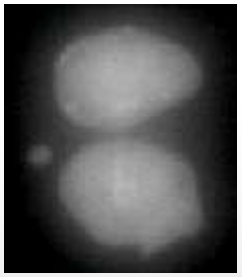


Max distance of nuclei in polynucleated cells

- The maximum edge-to-edge distance between nuclear structures that will be considered part of one cell. Any nuclear structures that are closer together than the value specified are grouped and are considered to be part of one cell.

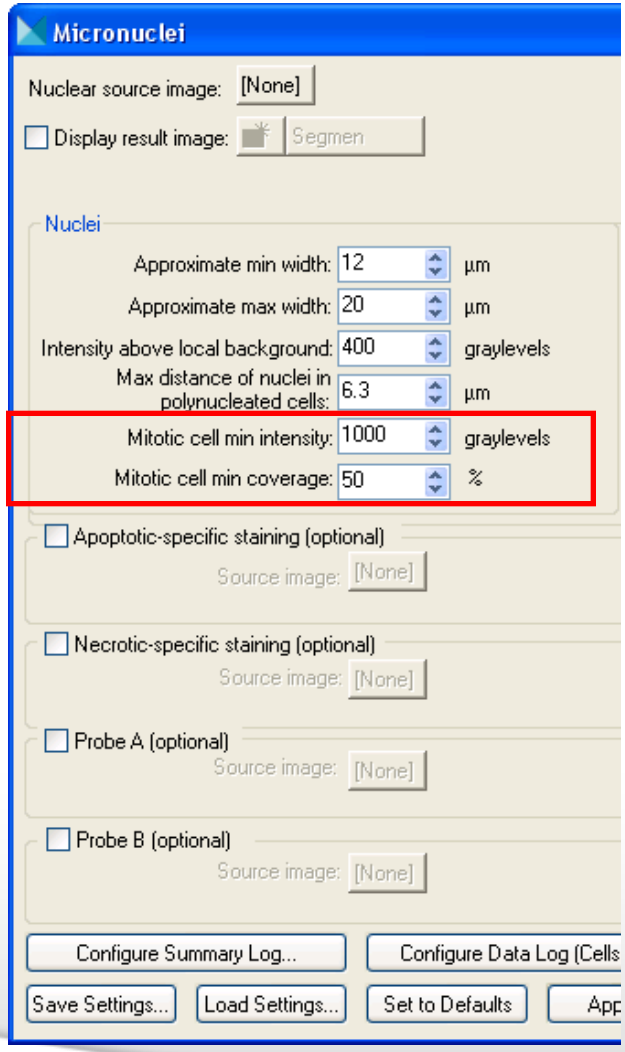


Mononuclear



Bi-nuclear

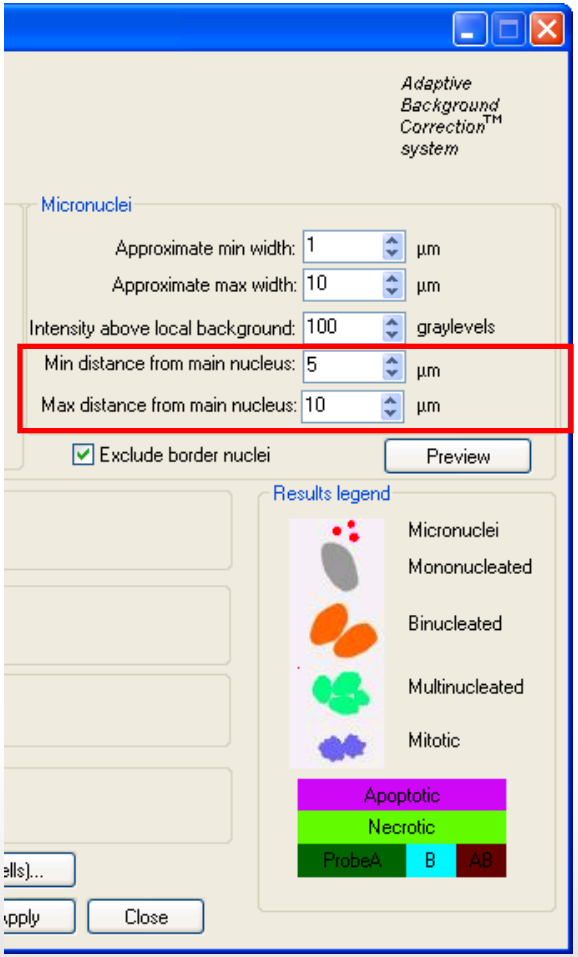
Module Settings



Mitotic cell min intensity and **Mitotic cell min coverage**

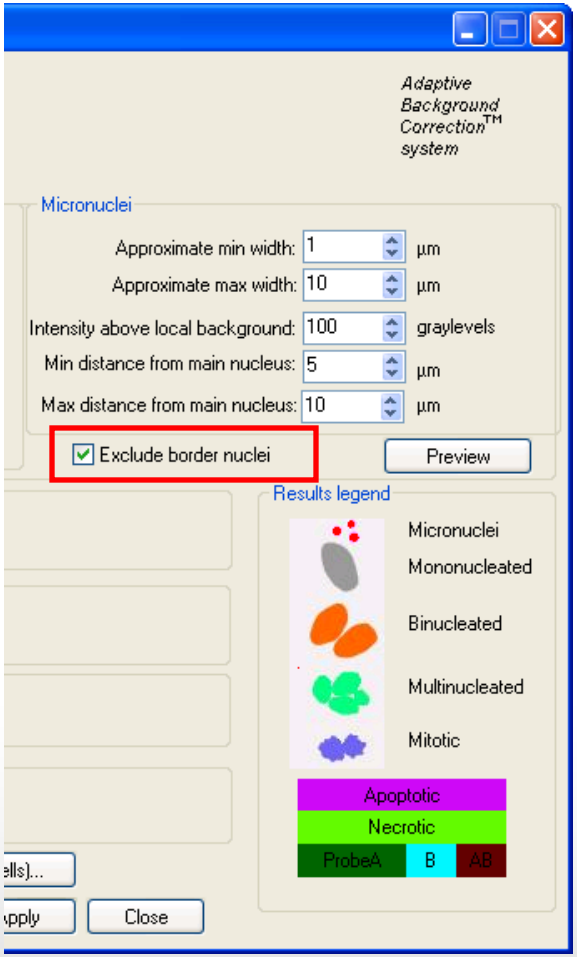
- To be classified as mitotic, a cell in the source image must meet the criteria that you specify in both of these fields.
- The minimum intensity is the intensity value of the dimmest mitotic cell nucleus in the field of view.
- The minimum coverage is the amount of a nucleus that has an intensity value equal to or greater than the Mitotic cell min intensity value that you specified. This helps ensure that a cell that has a small bright spot in its nucleus is not classified as mitotic.

2. Detect Micronuclei



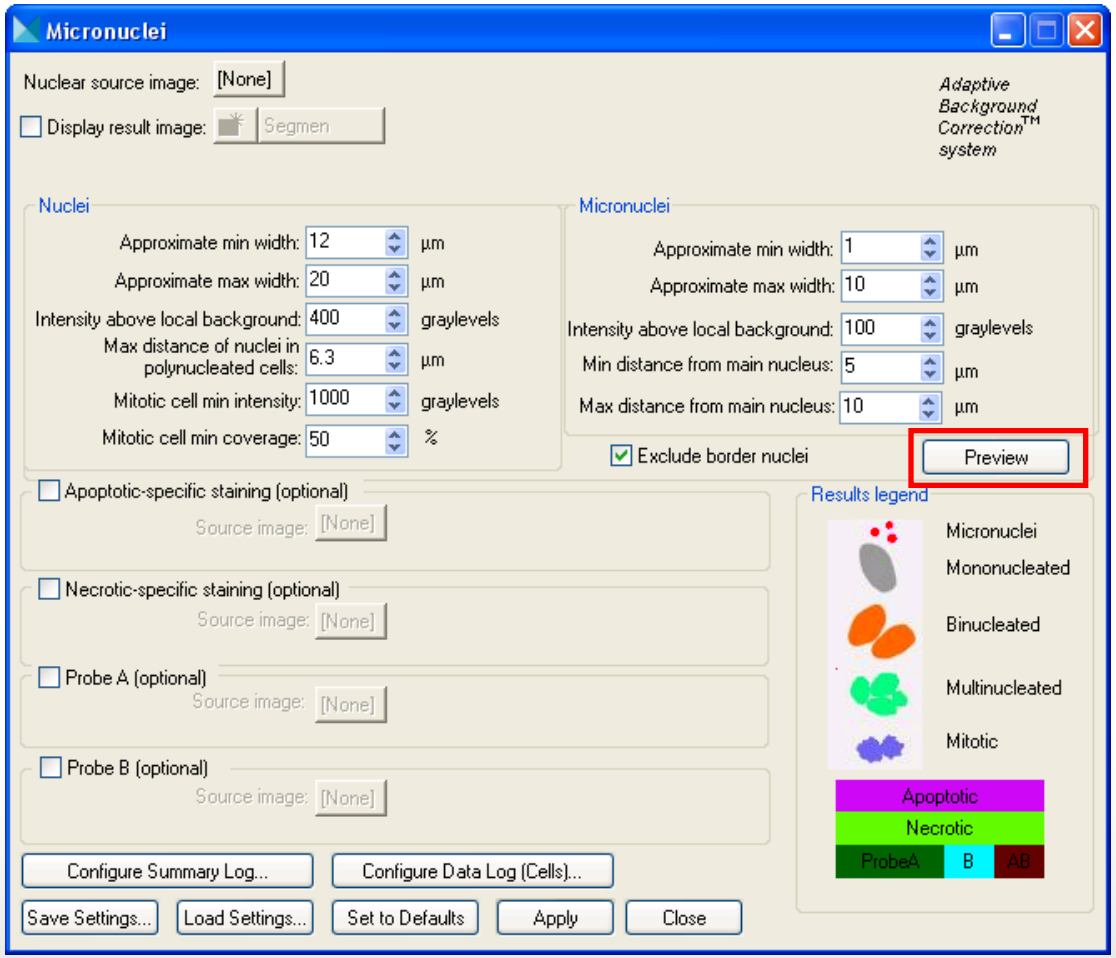
- **Nuclear stain**
- **Min distance from main nucleus** and **Max distance from main nucleus**
- The minimum and maximum distances that a micronucleus can be located from the cell's nucleus or nuclei.
- Any nuclear structures that are closer than the minimum or farther than the maximum specified distances to their main nuclei are excluded from the analysis.

2. Detect Micronuclei



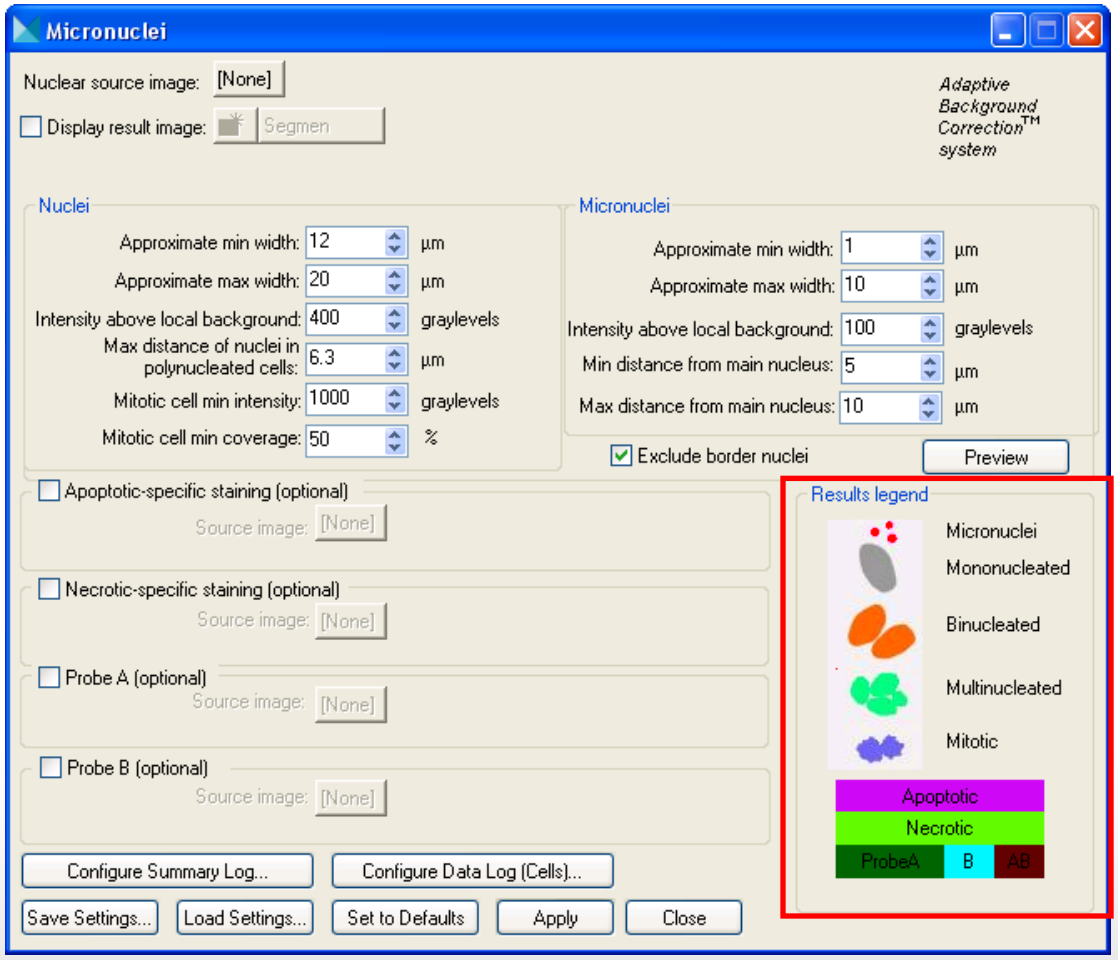
- **Exclude border nuclei**
- Specifies that the application should exclude from analysis any nuclei that touch the edge of the image.

Module Settings

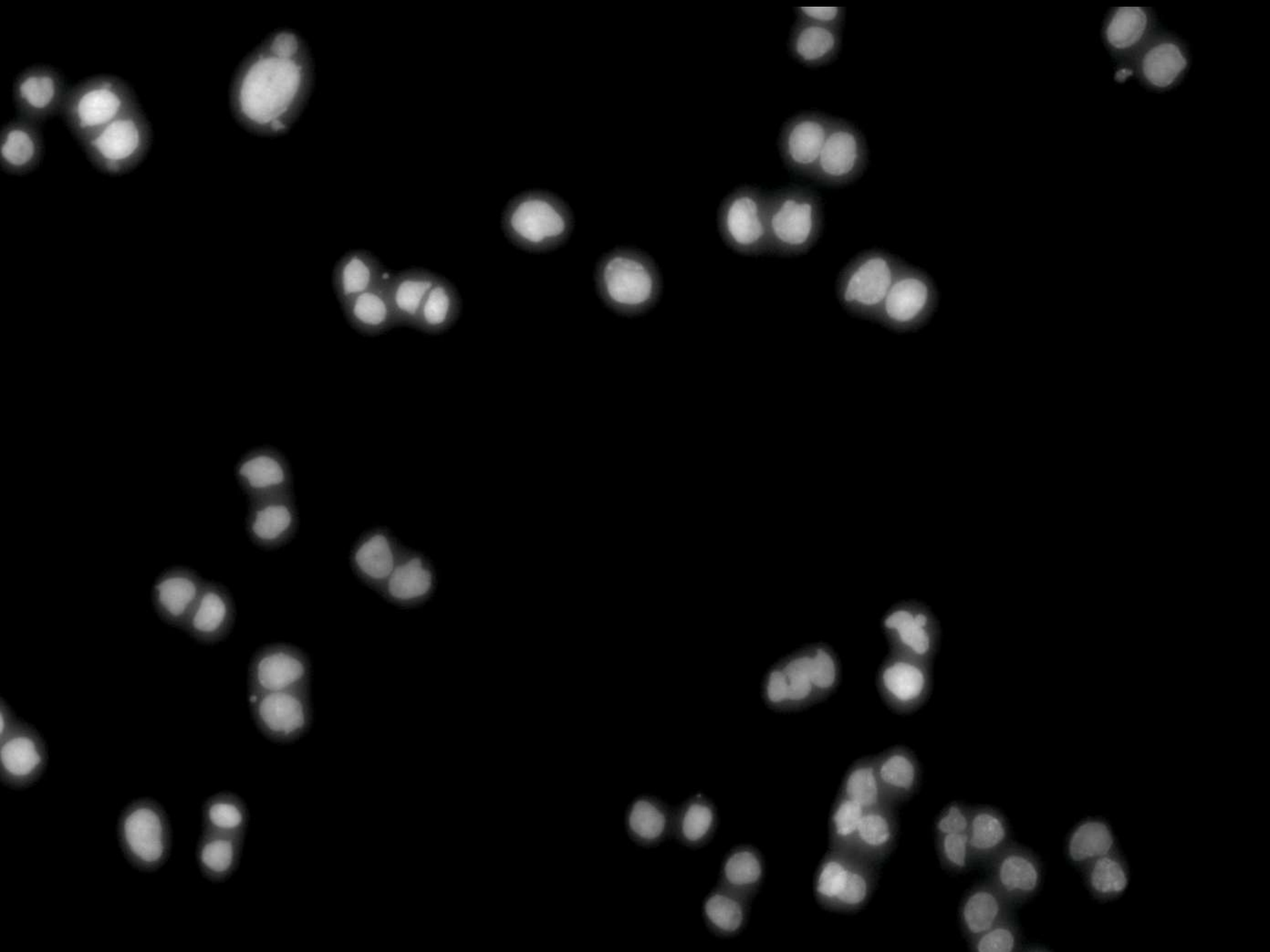


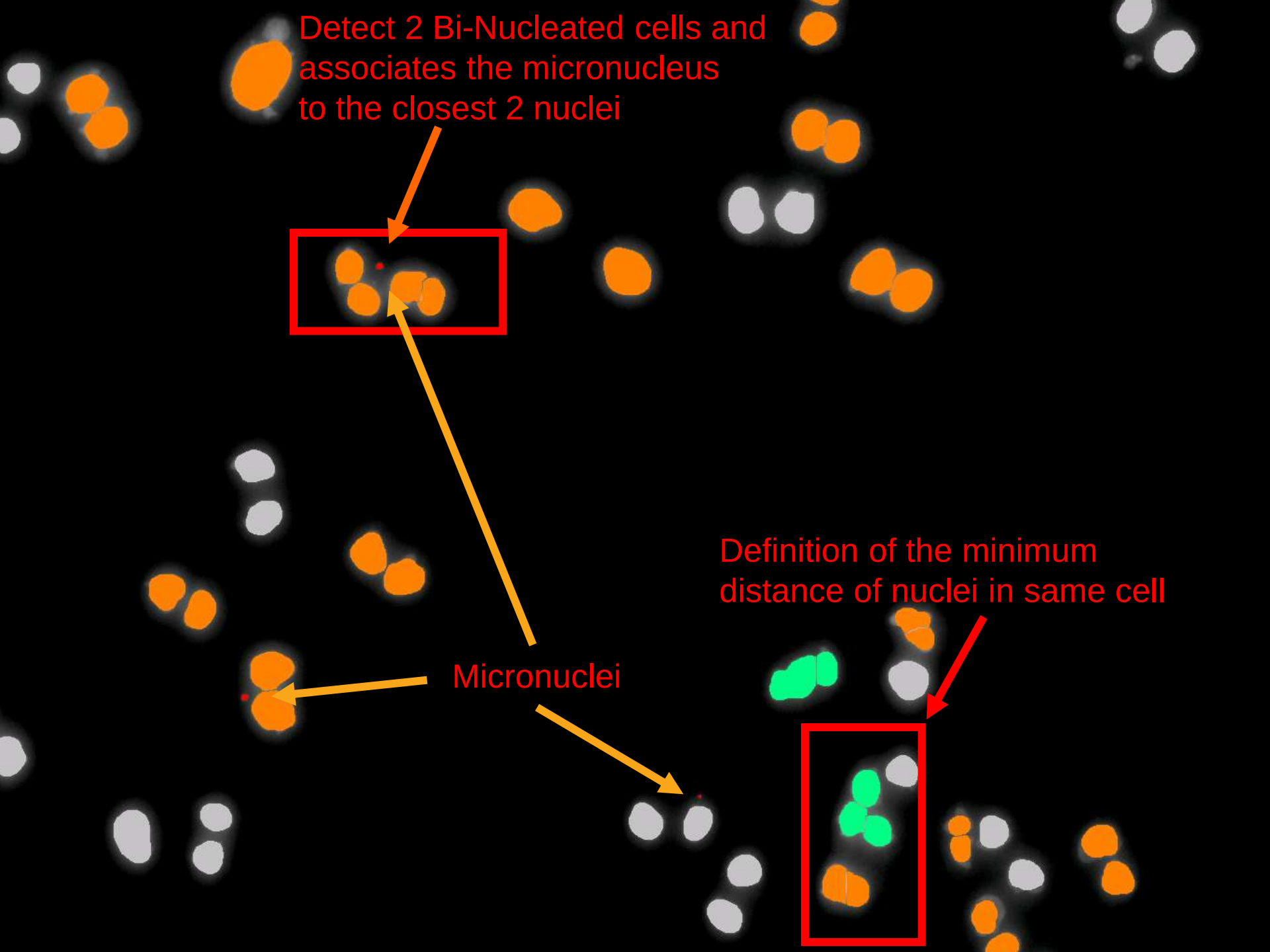
- **Nuclear stain**
- Click on **Preview** to test settings for the current wavelength only
- You can toggle between the preview overlay and the source image by clicking the Show/Hide Overlay tool in the source image window.

Module Settings

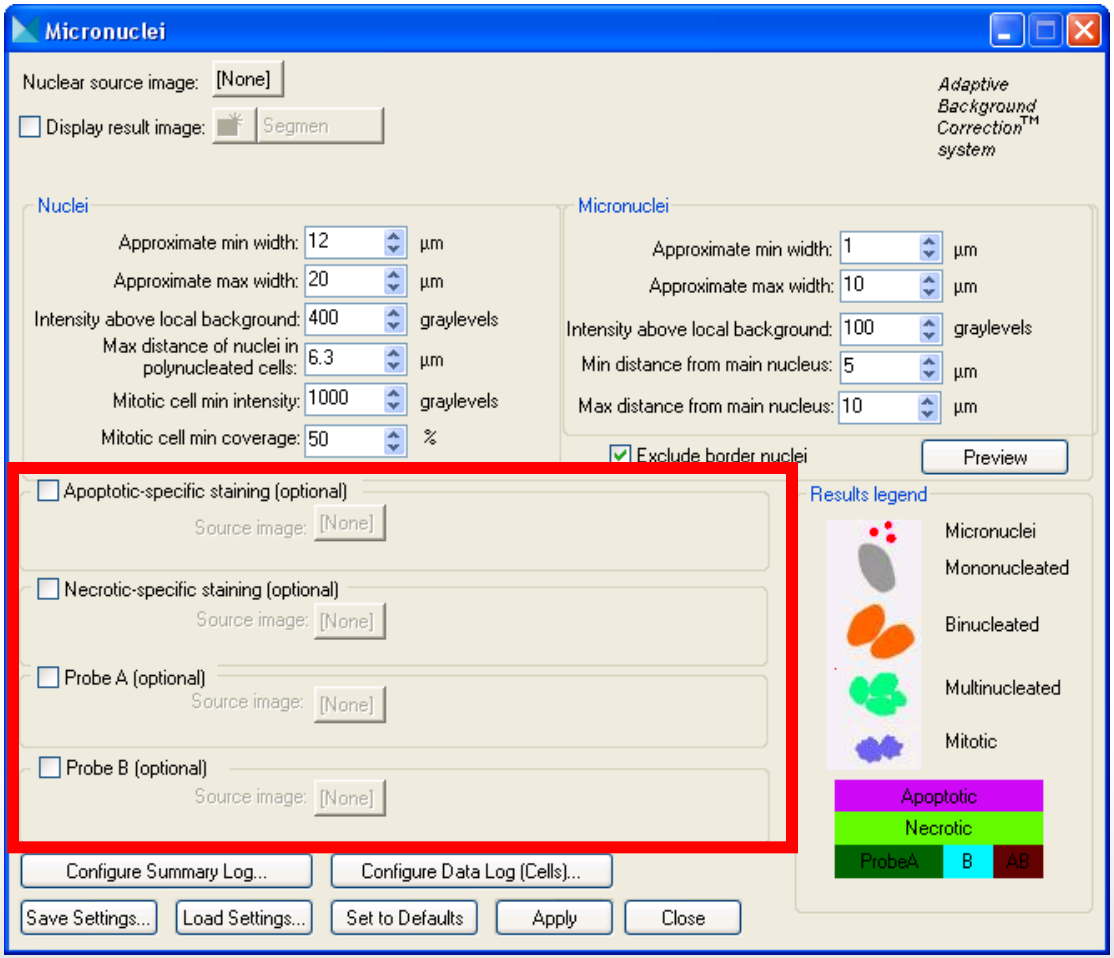


- **Results legend**
- The application module super-imposes an overlay with these color classifications on top of the source image.



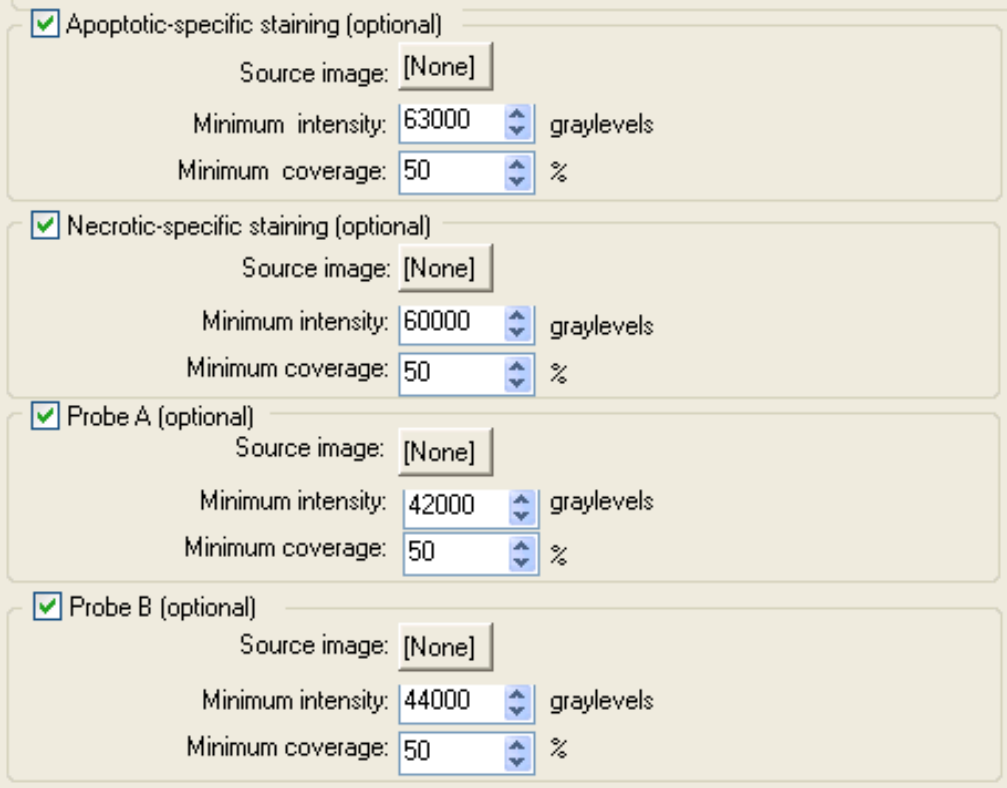

Detect 2 Bi-Nucleated cells and
associates the micronucleus
to the closest 2 nuclei
Micronuclei
Definition of the minimum
distance of nuclei in same cell

3. Multiplex with additional probes

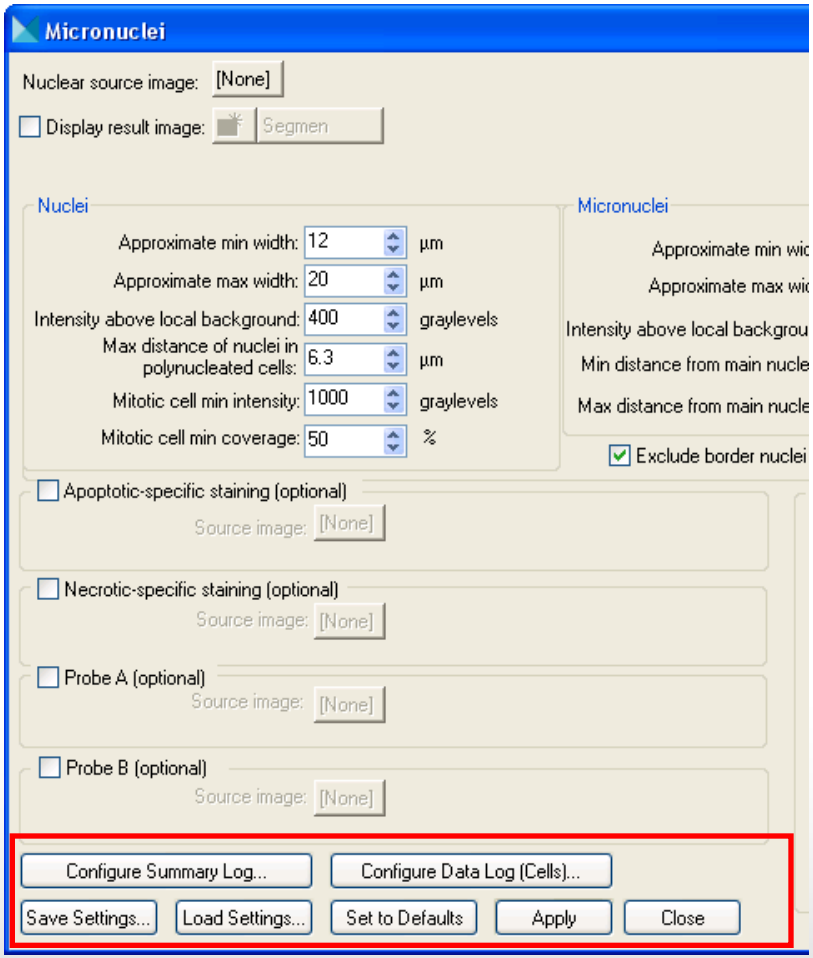


Multiplexing with additional probes

- Necrotic and apoptotic cells should not be included among the viable cells scored



4. Configure output



- **Configure Summary Log** – select site-by-site measurements
- **Configure Data Log** – select cell-by-cell measurements
- **Save Settings** – save analysis parameters to database
- **Load Settings** – load saved analysis parameters
- **Set to Defaults** – restore default analysis parameters
- **Test Run** – test all settings together and display cell-by-cell results for this site

Wide range of outputs

- 68 measurement per image, 30 measurement per cell
  - Healthy cells (one and 2 nuclei), Interphase, Mitotic, Mono/bi/multi-nucleated, Apoptotic, Necrotic, other additional probes
  - Sub-classification for Micronuclei for all cell classes: e.g. Mono-nucleated cells with one micronucleus vs. Mono-nucleated cells with multiple micronuclei
  - Area and intensities for all probes
  - Cell Division parameters: Nuclear division index, Dual/Mono-nucleated ratio, Multi/Mono-nucleated cells, (Multi+Dual)/Mono-nucleated
  - Etc.

Summary Data

Total Micronuclei	Total number of micronuclei for interphase cells
Micro-nucleated Cells	Total number of interphase cells with micronuclei
Cells with one micronucleus	Total number of interphase cells with one micronucleus
Cells with multi micronuclei	Total number of interphase cells with multi micronuclei
Micro and Mono-nucleated Cells	Total number of mono-nucleated cells with one or more micronuclei
Micro and Bi-nucleated Cells	Total number of bi-nucleated cells with one or more micronuclei
Micro and Multi-nucleated Cells	Total number of multi-nucleated cells with one or more micronuclei
Micro and ProbeA-positive Cells	Total number of ProbeA-positive cells with one or more micronuclei
Micro and ProbeB-positive Cells	Total number of ProbeB-positive cells with one or more micronuclei
Micro and ProbeAB-positive Cells	Total number of ProbeAB-positive cells with one or more micronuclei
% Healthy Cells	Healthy cells divided by total cells
% Mono-nucleated Cells	Mono-nucleated cells divided by total cells
% Bi-nucleated Cells	Bi-nucleated cells divided by total cells
% Multi-nucleated Cells	Multi-nucleated cells divided by total cells
% Micro-nucleated Cells	Micro-nucleated cells divided by total cells
% Cells with one micronucleus	Micro-nucleated cells with one micronucleus divided by total cells
% Cells with multi micronuclei	Micro-nucleated cells with multi micronuclei divided by total cells

68 total parameters for summary data of the well

Cellular Data

Cell: Necrotic	1 if necrotic, 0 if not
Cell: Mononucleated	1 if mononucleated, 0 if not
Cell: Binucleated	1 if binucleated, 0 if not
Cell: Multinucleated	1 if multinucleated (more than 2 nuclei), 0 if not
Cell: ProbeA Positive	1 if ProbeA positive, 0 if not
Cell: ProbeB Positive	1 if ProbeB positive, 0 if not
Cell: Number of Micronuclei	Number of micronuclei in this cell
Cell: DNA Area	Area (in calibrated units of measure) identified as nuclear or micronuclear
Cell: DNA Integrated Intensity	Integrated intensity over all pixels identified as nuclear or micronuclear
Cell: DNA Average Intensity	Integrated intensity over all pixels identified as nuclear or micronuclear divided by nu... corresponding pixels
Cell: Nuclear Area	Area (in calibrated units of measure) identified as main nuclear (includes bi- and m...
Cell: Nuclear Integrated Intensity	Integrated intensity over all pixels identified as main nuclear
Cell: Nuclear Average Intensity	Integrated intensity over all pixels identified as main nuclear divided by number of c...
Cell: Apoptotic Integrated Intensity	Integrated intensity over all pixels identified as main nuclear in an apoptotic cell

30 parameters for individual cellular data

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